THE ALIMENTARY CANAL OF ASAPHES MEMNONIUS HBST.

JOHN T. BIGHAM.

INTRODUCTION.

The material used in this work was collected by Dr. C. H. Kennedy at the Franz Theodore Stone Laboratory on Lake Erie, during the summer of 1929. The beetles were found feeding on watermelon rinds scattered in the forest. A considerable number of specimens were fixed in Kahle's Fixative, after which they were transferred to and preserved in 70% alcohol.

This species of click beetle is pale brown in color with paler legs. It varies in length from 13–26 mm. According to Horn it occurs from Canada to Georgia and westward to Colorado.

The author wishes to extend his thanks to Dr. Kennedy for the use of this material and also for helpful suggestions and criticisms during the progress of the work. He also wishes to acknowledge the help of Prof. J. S. Hine in determining the species.

GROSS ANATOMY OF THE DIGESTIVE TRACT.

General Discussion.

In insects the alimentary canal is divided morphologically into three primary regions on the basis of embryonic origin. The fore-intestine (stomodaeum) arises as an invagination of the ectoderm at the anterior end. The hind-intestine (proctodeum) arises as a similar posterior invagination. Connecting these two regions is the mid-intestine (mesenteron) which is derived from endodermal tissue. In correlation with the external epithelium the epithelial lining of the fore and hind intestines secretes chitin, which is found lining these two regions as a definite membrane.

The alimentary canal of *Asaphes memnonius* is of medium length as is usual in insects of phytophagus habit. In the region of the thorax it is incased by large wing and leg muscles. In other regions it is supported by tracheae and by suspensory muscles attached to the body wall.

Gross Structure of the Fore-Intestine.

In *Asaphes memnonius* the fore-intestine is very short. It extends from the mouth through the head capsule into the anterior part of the prothorax and comprises about one-thirteenth of the total length of the alimentary canal. It consists of two main divisions—*the pharynx* and *the crop*.

The pharynx begins at the mouth and comprises nearly the first half of the fore-intestine. In most specimens it appears as a narrow, nearly cylindrical tube, though in some specimens it is somewhat dilated. It has attached to it muscle strands which radiate out from it in various directions and are attached to the interior surface of the head capsule.

The crop varies considerably in size in different specimens. In some specimens there is no very distinct external line of demarcation between the pharynx and the crop. The crop continues on for some distance as a narrow tube of about the same diameter as the pharynx. Just before joining the mid-intestine it enlarges into a small bulb-like structure. In other specimens there is a constriction at the point of union of pharynx and crop and the crop begins to enlarge immediately and forms a sack two or three times as large as that in the specimens first mentioned. Since there is this variation in size, the crop apparently serves to some extent as a reservoir for food.

The Oesophageal valve marks the separation between the fore- and mid-intestine. The posterior end of the bulb-like crop disappears into the larger anterior end of the mid-intestine.

Gross Structure of the Mid-Intestine.

The mid-intestine is commonly known as the stomach. In this insect it is relatively long, extending from the anterior part of the prothorax to about the beginning of the third abdominal segment, and comprises approximately one-half the entire length of the alimentary canal.

It has a rather peculiar shape. The anterior portion lying in the prothorax and metathorax may be compared in shape to that of a club. The large end of the club is at the anterior end where it joins the fore-intestine. This enlarged end is somewhat flattened dorsoventrally, and it has shallow coeca which project forward on either side of and also dorsal to the posterior end of the crop. The number and position of these coeca seems to vary in different specimens. Following this enlarged anterior portion the stomach grows narrower and before it enters the mesothorax has become a narrow cylindrical tube. This tubular portion traverses the mesothorax. In the metathorax the stomach begins to enlarge again and forms a large pear-shaped sack which frequently occupies the larger part of the body cavity in this region. The shape of this enlarged posterior portion may be compared more exactly to that of a punching-bag used in gymnasiums. This enlarged portion extends into the second abdominal segment where it narrows abruptly, and the stomach once again becomes a tube which tapers towards its union with the hind-intestine at the pyloric valve, located at about the division between the second and third abdominal segments. The pear-shaped posterior enlargement of the stomach varies considerably in size and the tubular portions, both anterior and posterior to it, are correspondingly longer and shorter.

The surface of the stomach is covered with minute pimples which prove to be cell nests or nidi which project in part through the muscle layers of the stomach wall.

Gross Structure of the Hind-Intestine.

The hind-intestine comprises nearly one-half the total length of the tract. Its anterior limit is marked by the attachment of the malpighian tubules. It extends from this point to the anus.

The malpighian tubules are four in number and are attached at four different points around the circumference of the digestive canal, those on the two sides being slightly nearer together. They appear as long convoluted tubes which lie in the body cavity along the digestive tract. A few loops extend forward for a short distance to the pear-shaped portion of the stomach, but for the most part they run back and forth along the hind-intestine, in many places adhering to the walls of the intestine but not attached directly to it.

The pyloric valve lies just posterior to the attachment of the malpighian tubules. In this insect the pyloric valve is an unusually long structure occupying a section of the intestine which is about one-eighteenth of the total length of the hind-intestine.

The hind-intestine at the point where it joins the stomach is of the same diameter as the end of the stomach, but it immediately tapers down to form a narrow tube which makes a rather sharp bend to the right. This entire section of the intestine makes up the pyloric valve. From this point on the intestine has no very sharp divisions. The walls are very thin and the size and shape of different portions varies considerably, depending upon the amount and position of the food material contained.

At the end of the pyloric valve the intestine enlarges rather abruptly and extends cephalad to about the end of the first abdominal segment. Here it turns back again, thus forming a loop. The position in which this loop lies varies somewhat. The position shown in Fig. 1 is diagrammatic. It is usually not so wide a loop as this and often lies in such a position as to hide the pyloric valve. The portion of the intestine comprising the first half of this loop is usually comparatively narrow and the walls appear to have constrictions at more or less regular intervals. From the forward end of the loop back to the last segment of the abdomen the intestine is usually distended with food material and no constrictions are apparent. The walls of the intestine, especially in the dilated portion are very thin and are semi-transparent. When examined under a binocular, white muscle strands can be seen running longitudinally along the intestinal walls. In a few of the specimens examined the intestine was empty and appeared as a tube of about the same diameter from the pyloric valve to the last segment of the abdomen.

In the last abdominal segment the intestine becomes very much narrower and extends to the anus as a very narrow tube. Fig. 1 represents a female specimen. In the male specimens which were examined this portion of the intestine did not appear to be so narrow.

Histological Structure of the Alimentary Canal.

Histological Structure of the Fore-Intestine.

Although divided into two divisions, pharynx and crop, the fore-intestine reveals a uniformity of histological structure throughout its

length. The following layers of tissue from within outwards can be clearly demonstrated in its walls: (1) a primary intima, (2) a secondary intima, (3) an epithelium of hypodermal cells, (4) a layer of longitudinal muscles, and (5) a layer of circular muscles.

The intima is composed of chitin and is homologous with the cuticula of the body wall. It is a non-cellular, almost transparent layer and is secreted by the hypodermal epithelium. In the pharynx the two layers of the intima are fairly distinct. The primary intima stains darker but has a clear uniform structure. The secondary intima does not stain as dark and appears to have a granular structure. The intima of the pharynx is of nearly uniform thickness and presents a smooth even surface toward the lumen of the intestine. There are no spines present. In cross-sections of this region the intima is seen to be thrown up into several large folds.

In the crop the two layers of the intima are not very clearly differentiated. The division between them can be seen in some phases as an indistinct wavy line, the primary intima being relatively thinner than in the pharynx. The intima in the region of the crop is also much more irregular in appearance than in the pharynx. The side toward the lumen of the intestine presents a rough uneven appearance and in some places bears spines which are often long and needle-like. These spines are longer and more numerous near the esophagial valve. They seem to be distributed irregularly. In the narrow anterior portion of the crop the intima is thrown into numerous folds which nearly fill the lumen of the intestine. Where the crop is distended there are only a few small folds distributed at intervals.

The epithelium of the fore-intestine is a thin layer composed of irregular shaped cells. It is thrown into folds following the folds of the intima. In stained sections a continuous purple line can be seen along the edge of the epithelium on the side next the intima. The nature of this dark band is uncertain. The epithelial cells in most places are flattened and are much longer than thick, but in some places especially where the epithelium forms a fold they are cuboidal in shape.

In the crop the epithelium is even thinner than in the pharynx. In sections the epithelium appears in many places as a thin string with enlargements at intervals. One or more nuclei can be seen in most of these enlargements. The limits of the individual cells cannot usually be clearly distinguished except in the places where the cells are cuboidal in shape.

No basement membrane can be distinguished apart from the epithelial layer.

The logitudinal muscle layer of the fore-intestine is a continuous solid layer made up of a great number of bundles of muscle fibers. Nuclei are seen in many of the bundles, and are usually situated on the edges of the bundles as seen in cross-sections.

The circular muscle layer is a little thicker than the logitudinal layer. It is made up of rather thick muscle bands. Frequently there are three or four of these muscle bands running alongside each other. Numerous nuclei can be seen. They are often strung out in rows of four or five nuclei running lengthwise of the muscle. In longitudinal sections

of specimens in which the crop is of nearly uniform diameter throughout its length the circular muscle layer becomes thicker as it nears the oesophageal valve. Where the crop is distended the muscle layers are much thinner than otherwise.

In longitudinal sections in which the crop is distended up to the point where it joins the pharynx, the pharynx appears to project slightly into the lumen of the crop.

No "peritoneal membrane" was observed.

Histological Structure of the Oesophageal Valve.

The oesophageal valve marks the point of junction of the fore- and mid-intestine. Just anterior to the stomach the walls of the fore-intestine become constricted. Then they project into the lumen of the stomach, turn back upon themselves, and unite with the walls of the stomach.

The intima as it enters the constricted portion of the valve becomes smooth and regular in appearance. It passes into the stomach, turns back around the projecting lobe of epithelium and disappears between the lobe of fore-intestinal epithelium and the first group of stomach cells.

As the fore-intestinal epithelium enters the constricted portion of the valve its cells become distinctly cuboidal in shape. As it enters the stomach and turns back upon itself the cells become columnar in shape. Just after it makes the turn the cells become extremely long and narrow and appear closely packed together. A cleft usually appears between the last of these epithelial cells and the first of the stomach cells.

Longitudinal muscles are not especially abundant in the region of the valve. In some longitudinal sections the ends of the large longitudinal muscles of the stomach can be seen extending into the constricted portion of the valve.

The circular muscle layer of the fore-intestine becomes thicker just anterior to the valve and passing into the constriction at the valve it nearly fills that constriction with large masses of circular muscle bundles which by their contraction serve to close the valve.

At the oesophageal valve the muscle layers reverse their positions in respect to each other. Anterior to the valve the circular muscles are outside the longitudinal muscles, posterior to the valve the reverse is true.

Histological Structure of the Mid-Intestine.

The mid-intestine extends from the oesophageal valve to the pyloric valve. A histological examination of the stomach wall gives the following sequence of tissues from within out; (1) an epithelium of endoderm cells, (2) circular muscles, (3) longitudinal muscles. A basement membrane can probably be seen in some places at the base of the epithelial cells. No "peritoneal membrane" has been observed. The structure of the stomach wall appears to be about the same throughout its length although the diameter of cross-sections taken from its various portions varies greatly.

The appearance of the epithelial layer varies with the different stages in the process of secretion. The cells are columnar shaped but are much longer at some times than at others. Sometimes the cells form a single layer but usually they are built up into papillæ. Sometimes these papillæ become very tall and nearly touch one another on their sides. This is especially noticeable in the narrow portion of the stomach. Nidi or "cell nests" appear at regular intervals and are conspicuous objects since they stain darkly. These are nests of embryonic tissue from which new cells arise. They lie below the rest of the epithelium and project in part through the muscle walls. Since there is usually a papilla standing up between each two nidi, each nidus appears to be situated at the bottom of a pit.

Both merocrine and holocrine types of secretion seem to be carried on. In some cross-sections the food material in the intestine is seen to be completely surrounded with a ring of little globules of digestive secretion. These globules can be seen issuing from the ends of the epithelial cells. Apparently the cells rupture at their ends and the fluid secretion contained in the extremity of each cell flows out forming a globule as it enters the lumen of the intestine. The cell wall at this emptied end then shrinks and becomes wrinkled and shrivelled in appearance. This gives the ends of the cells a striated appearance. At an apparently later stage the cells are seen to be tipped with filaments which are probably the frayed out walls of the cell ends which have just been described. Among the cells of this second stage can be seen some which show at their tips a convex surface within the filaments. This probably is the surface of new secretion which is welling up from the body of protoplasm below. This second secretion is thrown off much as in the first case. Apparently this process repeats itself as many as two or three times. In the first stage the cells formed a single layer. In the mean time they have been building up and forming papillæ. The nuclei gradually move out towards the tips of the cells and finally the entire mass of cells breaks down in the form of holocrine secretion. This latter process was plainly taking place in many of the sections which were examined. New cells then grow out from the nidi and replace the old ones.

The process which has just been described as the probable method of secretion in this insect was suggested to the author by Dr. C. H. Kennedy.

Just outside the epithelial layer are large circular muscle bundles. These bundles are more or less isolated and do not usually form a continuous layer. In some places, however, their ends overlap and they appear in cross-section as a nearly continuous ring around the epithelium.

Outside the circular muscles are the longitudinal muscles. These also occur as isolated bundles, thirty or forty or more of them being seen in a cross-section. Frequently several of these bundles are seen grouped together.

Between the epithelial layer and the circular muscles especially within folds of the epithelium, fine strands of tissue appear which are probably tracheoles. In such places there can be seen also apparently isolated nuclei. The nature of these is uncertain.

Histological Structure of the Hind-Intestine.

The malpighian tubules arise as outpocketings of the hind-intestine. In *Asaphes memnonius* they join the digestive tract just at the point of junction of the mid- and hind-intestine. The epithelium just anterior to the insertion of these tubules is composed of typical stomach cells while the epithelium immediately posterior to their insertion is covered with a thin layer of chitin which shows that it belongs to the hind-intestine.

The epithelial cells of the tubules are very irregular both in shape and size. Their irregular shape gives the lumen of the tube a very irregular outline. Frequently two large cells will have very small cells inserted between them. The nuclei are large and usually ovate in shape. The tubules are lined with a striated border. This border looks as if it were made up of a dense lining of fine filaments extending out from the epithelial cells and with a comparatively few longer filaments extending out beyond the tips of the others.

At the origin of the tubules the epithelial cells appear crowded together and columnar in shape. Progressing distally from the basal end of the tubule the cells gradually assume their more characteristic appearance.

On the outside the Malpighian tubules are bounded by a narrow layer of connective tissue containing many nuclei.

The pyloric valve is situated just posterior to the point of insertion of the malpighian tubules. In *Asaphes memnonius* this valve is an unusually long structure occupying the entire length of the first bend in the hind-intestine.

Immediately posterior to the insertion of the malpighian tubules the epithelial layer is thrown into a number of folds surrounding the lumen of the valve. The cells making up these folds are long and columnar shaped. As the epithelial layer passes from this point into the constricted portion of the valve the cells become cuboidal and finally small and irregular in shape, giving the epithelial layer somewhat the same appearance as the fore-intestinal epithelium. Throughout the constricted portion of the valve the epithelium is thrown into long folds running lengthwise of the valve. Near the posterior end of the valve where it is usually tightly constricted these folds practically obliterate the lumen of the valve. The epithelial layer is covered with an intima of chitin. The intima is very thin at the anterior end of the valve but becomes thicker toward the posterior end. At the point where the pyloric valve ends and is followed by a wider portion of the intestine the cells of the epithelium become much larger and assume rather suddenly the characteristic cuboidal shape of the cells of the hind-intestine. The end of the valve frequently projects slightly into the lumen of this portion of the intestine.

The pyloric valve is surrounded for its entire length by a conspicuous layer of circular muscles, and there is an especially dense mass of these muscles at the point where the valve makes a bend. Evidently the muscles serve to close the valve at this point.

Longitudinal muscles are found both outside and inside the circular muscle layer.

The hind-intestine proper begins at the end of the pyloric valve and extends to the anus. The intestinal walls throughout this region show a close similarity of structure. The tissues are arranged in the following manner: (1) an inner intima of chitin, (2) an epithelial layer, (3) circular muscles, and (4) longitudinal muscles. In a few longitudinal sections taken from the anterior part of the hind-intestine there appeared to be some circular muscle bundles outside the longitudinal muscles.

As was mentioned in the description of the gross structure, the hind-intestine proper appears externally to be divided into three regions, a narrow convoluted anterior portion, an enlarged median portion, and a narrow posterior portion. Although differing in diameter these regions do not appear to be greatly different in morphological structure. The walls of the anterior portion appear to be constricted. The epithelium is thrown up into folds as seen in both longitudinal and cross-sections. The epithelium of the narrow posterior portion is convoluted also. In the enlarged median portion the walls appear to be stretched and no convolutions appear. In some specimens in which the digestive tract was empty the hind-intestine appeared as a tube of nearly uniform diameter throughout its length. However, it appears that the median portion is the largest portion aside from the fact that its walls are usually distended.

The intima of the hind intestine is a thin layer of chitin adhering closely to the epithelial layer.

The epithelial layer is composed of cells which are usually columnar shaped although they are comparatively short and thick and many of them would be described as cuboidal. The nuclei are comparatively large ovate structures situated usually nearer the basal portion of the cells. When stained with eosin the outer ends of the cells stain darker than their basal portions. This gives the appearance of a dark band along the side of the epithelium next the lumen of the intestine. In places this band appears somewhat striated.

The circular muscles are found immediately surrounding the epithelium. They are numerous but do not usually form a solid layer. In places where the intestine is constricted there will be frequently two or three of these muscle bands running alongside each other.

The longitudinal muscles are outside the circular muscles. They are larger than the circular muscles but not so numerous. In cross-sections they appear in groups scattered around the circumference of the section.

Where the epithelium is convoluted, both longitudinal and circular muscles are found running up into the folds in some places.

The muscles are striated as are all insect muscles. When they are contracted their edges have a beaded appearance.

BIBLIOGRAPHY.

1. **Blatchley, W. S.** 1910. Coleoptera of Indiana.
2. **Fletcher, F. W.** 1929. The Alimentary Canal of Phyllophaga gracilis Burm. Ohio Jr. Sci., Vol. XXX, No. 2.
3. **Horn, G. H.** 1880. Notes on the Species of Asaphes of Boreal America. Trans. of the Amer. Ent. Soc., Vol. VIII, p. 74.
4. **Jahn, L. A.** 1929. The Internal Anatomy of the Mydas Fly. Ohio Jr. Sci., Vol. XXX, No. 2.
5. **Landis, B. J.** 1929. The Alimentary Canal of Megilla fuscilabris, Muls.
6. **Packard, A. S.** 1903. A Textbook of Entomology.

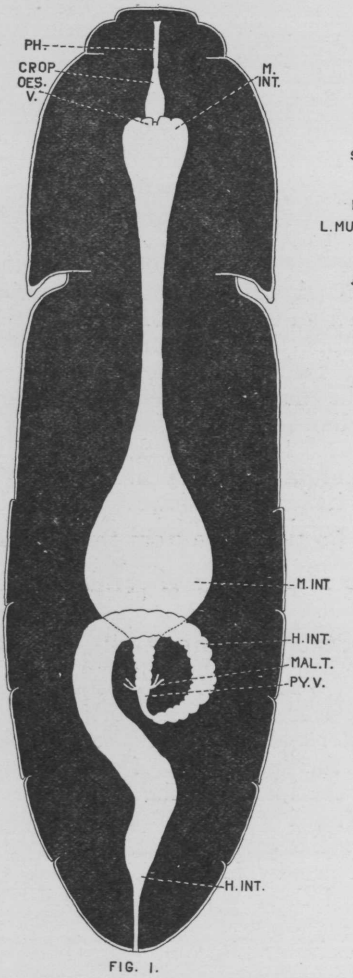

FIG. 1.

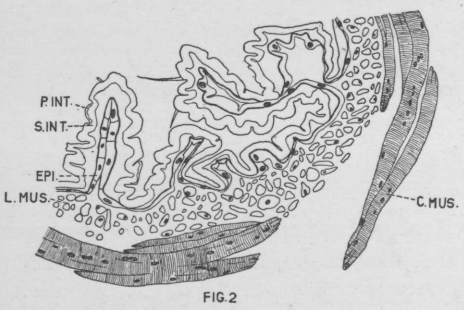

FIG. 2

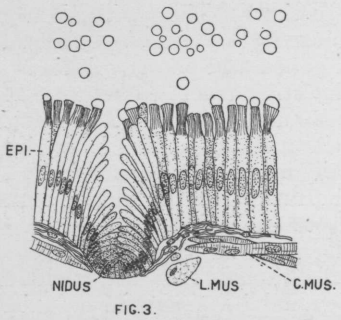

FIG. 3.

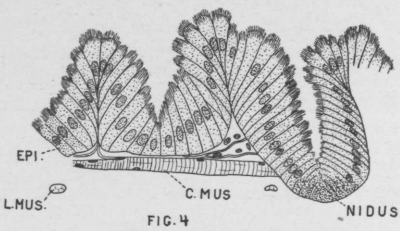

FIG. 4

Fig. 1. A dorsal view of the alimentary canal of *Asaphes memnonius*.
Fig. 2. Cross-section through constricted anterior portion of crop.
Fig. 3. Cross-section through mid-intestine showing "first stage" in the process of secretion.
Fig. 4. Cross-section through mid-intestine showing a later stage in the process of secretion.

KEY TO ABBREVIATIONS.

C. Mus.—Circular muscles.
Epi.—Epithelium.
H. Int.—Hind-intestine.
Int.—Intima.
L. Mus.—Longitudinal muscles.
Mal. T.—Malpighian tubule.
M. Int.—Mid-intestine.
Oes.—Oesophagus.
Oes. V.—Oesophageal valve.
Peri. mem.—Peritoneal membrane.
P. Mem.—Peritoneal membrane.
Ph.—Pharynx.
P. Int.—Primary intima.
Py. V.—Pyloric valve.
S. Int.—Secondary intima.

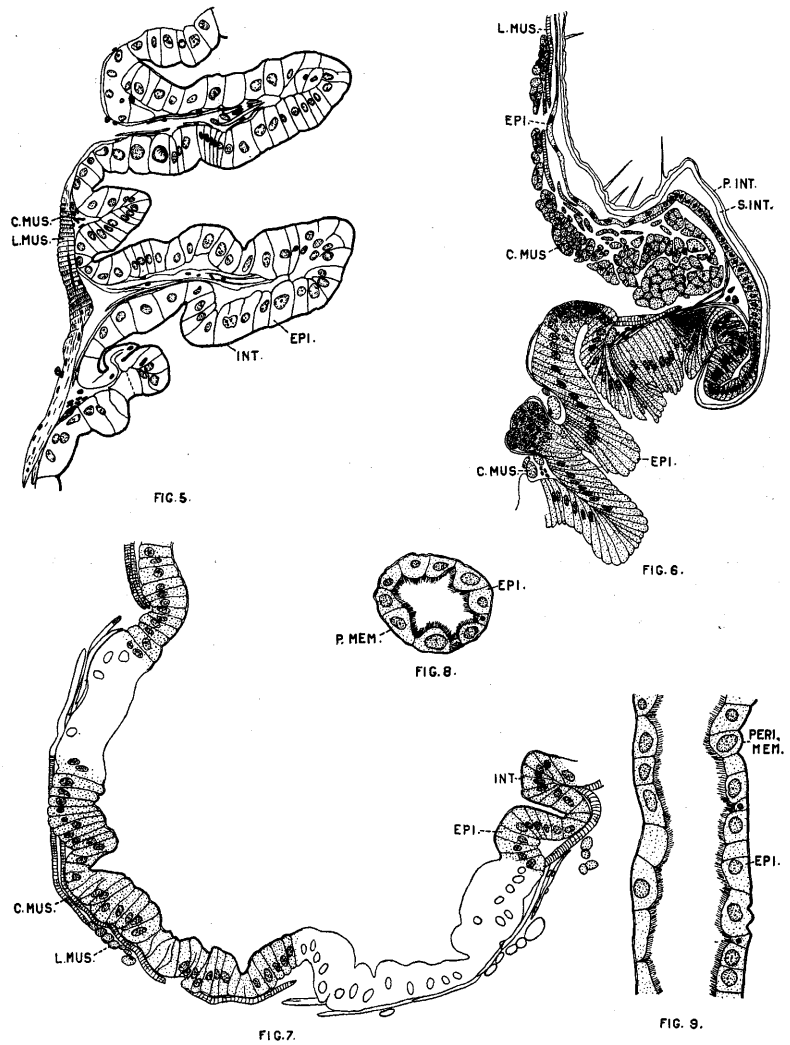

Fig. 5. Longitudinal section through anterior convoluted portion of hind-intestine.
Fig. 6. Longitudinal section through oesophageal valve.
Fig. 7. Cross-section through posterior portion of hind-intestine.
Fig. 8. Cross-section of Malpighian tubule.
Fig. 9. Longitudinal section through Malpighian tubule.

NOTE: Figures 5, 6 and 7 are magnified approximately five-eighths as much as the other histological figures.